Footprinting of the Hairpin Ribozyme

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Our studies demonstrate that the hydroxyl-radical footprints of the hairpin ribozyme-substrate complex observed using Fenton chemical reagents (Hampel etal., 1998), can be perfectly reproduced using the synchrotron footprinting method. We have now completed an initial survey of footprinting kinetics for the entire hairpin ribozyme using the native form of this catalytic motif. The rates for folding of all protected site of the complex are faster than 20 s $^{-1}$ in both $\text{Co(NH}_3)_6^{3+}$ - and Mg^{2+} - containing buffers. We have not been able to define the folding rates precisely since all sites on the ribozyme become completely protected during the deadtime of the footprinting assay (100 ms). Since folding of the ribozyme-substrate complex has a considerable activation energy, we will attempt to slow folding by lowering the temperature of the footprinting experiments. In addition, the high signal to noise ratio of previous experiments at 100 ms beamline exposure suggests to us that it will be possible to directly decease the deadtime of the experiments.